Photoionization and excitation processes in proteins and peptides

Egorov, Dmitrii

Chapter 6

Summary

The molecular understanding of the interaction of ionizing radiation with proteins and peptides is of great importance to astro- and radiobiology, various soft X-ray microscopic techniques and future peptide secondary and tertiary structure research. In this thesis we have performed systematic studies of molecular fragmentation patterns of peptides and proteins and their non-dissociative ionization yields as a function of molecular size and conformation. The molecular ionization and fragmentation processes have been triggered by absorption of energetic photons of 10-500 eV, i.e VUV and soft X-ray photons. The main conclusions for every chapter are the following:

In Chapter 3 we presented the results of near-edge X-ray absorption mass-spectrometry of peptides and proteins of various masses for two photon energies, one resonant with the 1s-$\pi^*_{C=O}$ transition and the other one well above the ionization threshold. In both cases an inner shell C (1s) vacancy was created, which was subsequently refilled in an Auger process in which two valence electrons participate. One of the valence electrons decayed to the C (1s) vacancy while simultaneously the second valence electron was emitted from the molecule. The Auger process increased the charge state of the molecule by 1. Therefore for resonant 1s-$\pi^*_{C=O}$ excitation and ionization the initial charge state of the molecule was raised by 1+ and 2+, respectively.

The molecular masses ran from 500 Da to more than 12 kDa. The near-absence of backbone scission peaks in the fragmentation spectra obtained upon soft X-ray absorption made them distinctly different from what is observed with conventional methods. For the smaller molecules up to approximately 2kDa the fragment mass spectra were dominated by small fragments while for larger molecules with masses exceeding ~8 kDa the prominent mass-spectral features were due to non-dissociative ionization. Molecular fragmentation was not predominantly driven by the increase of charge state but by the amount of energy deposited into the system. The deposited energy was defined by the Auger process which generates two holes in the valence shell. The average amount of deposited energy was in the range of 15-25 eV. In the framework of a simplified harmonic oscillator model, the deposited excitation energy was transformed into a peptide/protein internal temperature. Molecular fragmentation was observed to

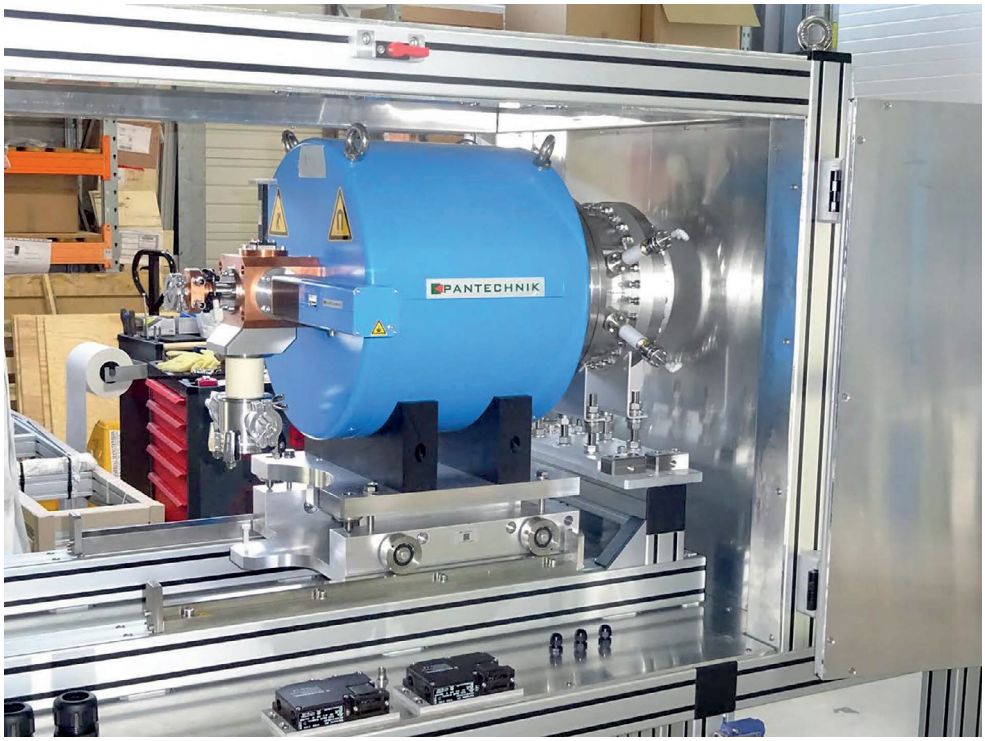

Figure 6.1. Supernanogun, ECRIS source employed by our group.

increase strongly with internal temperature, with the onset being at lower temperature than what is commonly observed with conventional mass spectrometric techniques such as CID, SID, and direct heating based on vibrational excitation of the molecular system. The temperature ranged from 413 K in system with the size of 12 kDa to 1678 K in the system with size of 0.5 kDa.

Most likely, the reason for the differences lay in the competition of IVR and fast local fragmentation after the Auger process.

The obtained quantitative relations between fragmentation and non-dissociative ionization yields and protein temperature were in agreement with earlier research of protein laser desorption and thermal desorption, though the qualitative picture of thermal desorption spectra differed from soft X-ray absorption spectra mainly by higher abundance of backbone scission fragments and lower abundance of immonium ions.

In Chapter 4 we performed a similar study on peptides but now 20 eV VUV photons were used. The 20 eV photons were directly absorbed by peptide. As only a single vacancy was created in the valence shells of the peptides, less energy was deposited as compared to soft X-ray absorption and the transition from the

extensive fragmentation to the non-dissociative ionization regime occured at much smaller molecule size. However, despite the fact that there was a general transition from dissociative ionization of very small peptides to non-dissociative ionization of larger systems, the relation between non-dissociative ionization and molecular size was not monotonic for the molecules under study here. This deviated from our previous observations for the soft X-ray range. This meant that in many systems effects such as molecular conformation and protonation state can more than compensate the pure statistical fragmentation.

Complementary C- and N-terminal fragments were observed for angiotensin, melittin and gramicidin A. Most likely protonation sites were discussed and in particular for melittin the location of the photoinduced charge were determined.

Additionally, the influence of the photon energy on the photofragmentation patterns was studied. For the example of $[\text{gramicidin A+2H}]^{2+}$, photofragmentation spectra for 14, 20 and 35 eV showed the influence of a transition from predominant photoexcitation to deep valence photoionization on the obtained mass spectra. The fingerprint of this process was the transition of several large singly charged sequence ions to the corresponding doubly charged state. The relative yield of immonium ions strongly increased with photon energy, while non-dissociative ionization increased from 14 eV to 20 eV and decreased when E_{ph}=35 eV. For 14 eV the contribution of excitation without ionization was high. This obviously led to a lowered fraction of non-dissociative ionization, while for 20 eV the contribution of excitation without ionization was low. For 35 eV inner valence electrons became accessible. Accordingly, the average energy deposited in the system increases, leading to an increase of dissociation.

From the point of view of the experiments conducted in chapters 3 and 4 of this thesis it would be interesting to check if the dependency of peptide and protein fragmentation on their size upon absorption of VUV and soft X-rays also exists for energetic ions. Via electronic stopping (energy loss) processes energetic ions interacting with a molecule interact mainly with the valence electrons and thus deposit energy in the valence shells similar to VUV and soft-X-ray absorption. The energy loss is proportional to the velocity of the ions. To investigate the similarities between photons and ions a scan of non-dissociative ionization yields on molecule size and ion energy should be made. The ion beams can be provided by the Supernanogan ECRIS source (Pantechnik, France), see Figure 6.1, recently installed at the Zernike Institute for Advanced Materials. The ion experiments might be of relevance to a molecular understanding of new proton and hadron based radiotherapy.

In Chapter 5 we performed a soft X-ray absorption spectroscopy case study for the melittin peptide. Three main classes of reaction channels analyzed in this chapter

were: non-dissociative ionization, small neutral molecule loss and formation of ions due to backbone scission.

The analysis of non-dissociative ionization yields showed substantial double ionization of protonated melittin *below* the ionization threshold. This was explained by (secondary) electron impact ionization by Auger electrons emitted after soft X-ray absorption. We observed a clear decline of non-dissociative double ionization below the ionization threshold with increasing melittin protonation state. This was explained by the lower probability of secondary electron ionization by the Auger electrons due to the less compact structure of melittin for higher charge states. This explanation was underpinned by Monte Carlo simulations of secondary electron ionization in the framework of the independent atom model (IAM).

A prominent loss channel that was observed was the loss of neutral molecules. Interestingly, in the case when buffer gas was applied during the peptide irradiation, all these channels were quenched with the exception of the channel in which 44 mass units are lost, i.e a loss channel related to arginine. This implied that this channel has the lowest activation energy or the highest dissociation rate.

Yields of backbone scission ions were fitted with superpositions of non-dissociative single (NDSI) and double ionization (NDDI) spectra. The ratio between the NDSI and NDDI in the fit is defining the relative importance of single and double ionization for the respective channel. Deviations between the NDSI+NDDI fit and the actual fragment yields as a function of photon energy were mainly observed in the 290-295 eV energy range, where experimental data was exceeding the fit for some fragments. This difference was explained by the higher activation energy for formation of these fragments.

The obtained results on melittin have provided a deep insight into the interaction of energetic photons with peptides. In addition to that, experimental methodology allowing for the assessment of the structure of gas-phase peptides was developed.